Thermodynamic Study of Native Tyrosinase and its Modification Form by Diethyl Pyrocarbonate

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Abstract

Mushroom Tyrosinase (MT. EC. 1.14.18.1) is a poly phenol oxidase enzyme with two copper in active site involved in the formation of the pigments of skin, hair and eye in mammalian. The thermodynamic parameters of Mushroom Tyrosinase (MT) was investigated in presence of histidin-specific modifier reagent Diethyl pyrocarbonate (DEPC) after thermal and chemical denaturations. The denaturations of MT were assessed in the sole enzyme and its modified form in the presence of 0.5, 1, 5 and 10 mM concentrations of DEPC. The thermal denaturation of the enzyme the Tm (melting point) and ΔG_{25}°C (Gibbs energy) values have been obtained in native and modified forms. The Tm and ΔG_{25}°C values of thermal denaturation for MT were determined 53, 50, 43.2, 42, 41.5 C°, and 6.6, 4.1, 3, 2.5, 2 kJ/mol, in the DEPC concentrations of 0, 0.5, 1, 5 and 10 mM respectively. In the chemical denaturation by Guanidium Hydrochloride (8 M), the magnitudes of Cm (half of modifier's concentration) and ΔG_{H2O} (free energy) values for enzyme have been obtained 2, 1.9, 1.8, 1.7, 1.5 M and 8.4, 7.3, 6.9, 6.2, 5.4 kJ/mol, in the DEPC concentrations of 0, 0.5, 1, 5 and 10 mM respectively. The overall comparison of thermodynamic parameters between native and modified form of MT showed instability of modified by DEPC and emphasized to the crucial role of hitidine residues in the enzyme structure.

Keyword: Mushroom tyrosinase, Diethyl pyrocarbonate, Thermal, Chemical, Denaturation